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Vinyl chloride-induced hepatic lesions in man and rodents. A comparison

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ABSTRACT – Histologic sequences in the liver of rodents exposed by inhalation to gaseous vinyl chloride were compared to the lesions in man exposed to the same agent, mainly in vinyl chloride polymerization plants. An identical sequence, starting with circumscribed proliferation of hepatocytes, soon followed by proliferation of a variety of sinusoidal cells and frequently associated with sinusoidal dilatation, progresses to intralobular and more frequently to trabecular angiosarcoma. Predominantly in young animals and rarely in man, hepatocellular carcinoma develops, but never cirrhosis. The sequence represents a dynamic process of competition between proliferating hepatocytes and sinusoidal cells, of hepatocytes with fibroplasia, between perisinusoidal fibrosis and sinusoidal dilatation, and of proliferation of various sinusoidal cells versus angiosarcoma. The great similarity in the evolution in man and rodents, rarely encountered in other experimental models, supports the prediction of human cancer from animal experiments. The precursor nodules differ from the nodules commonly observed in hepatocarcinogenesis by co-proliferation of sinusoidal cells. The differences in the reactions between man and rodents bespeak a strong fibroblastic reactivity in man. Most important, the precursor lesion of mixed hepatocellular and sinusoidal cell proliferation may be of diagnostic value, being superior to conventional hepatic tests in detection of some initial environmental lesions.

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Less than ten years ago, the relation between vinyl chloride and hepatic angiosarcoma, a rare human tumor, became apparent when the tumor was discovered in a relatively small work force in plants in which gaseous vinyl chloride was polymerized to polyvinyl chloride (1). This finding was preceded by the observation (2) that prolonged inhalation of gaseous vinyl chloride produced in rodents various tumors, of which hepatic angiosarcoma was outstanding. Subse-

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quent study of extensive material of human angiosarcomas following exposure to vinyl chloride demonstrated a sequence (3, 4) from a hepatic precursor lesion frequently associated with portal hypertension and its common sequelae, such as esophageal variceal bleeding (5, 6), to angiosarcoma. By now, worldwide, less than 100 instances of hepatic angiosarcoma have been associated with vinyl chloride exposure. The identical sequence was, however, found also in man exposed therapeutically (7, 8) or occupationally (9) to inorganic arsenicals or to androgenic/anabolic steroids (10) and after administration of thorotrast for roentgenographic purposes (11), but, furthermore, in persons without established etiology, in whom, therefore, unknown environmental factors were incriminated (4). One instance of copper-related angiosarcoma was reported (12), and there are rare instances of association of angiosarcoma with hemochromatosis of unknown etiology (13, 14, 15).

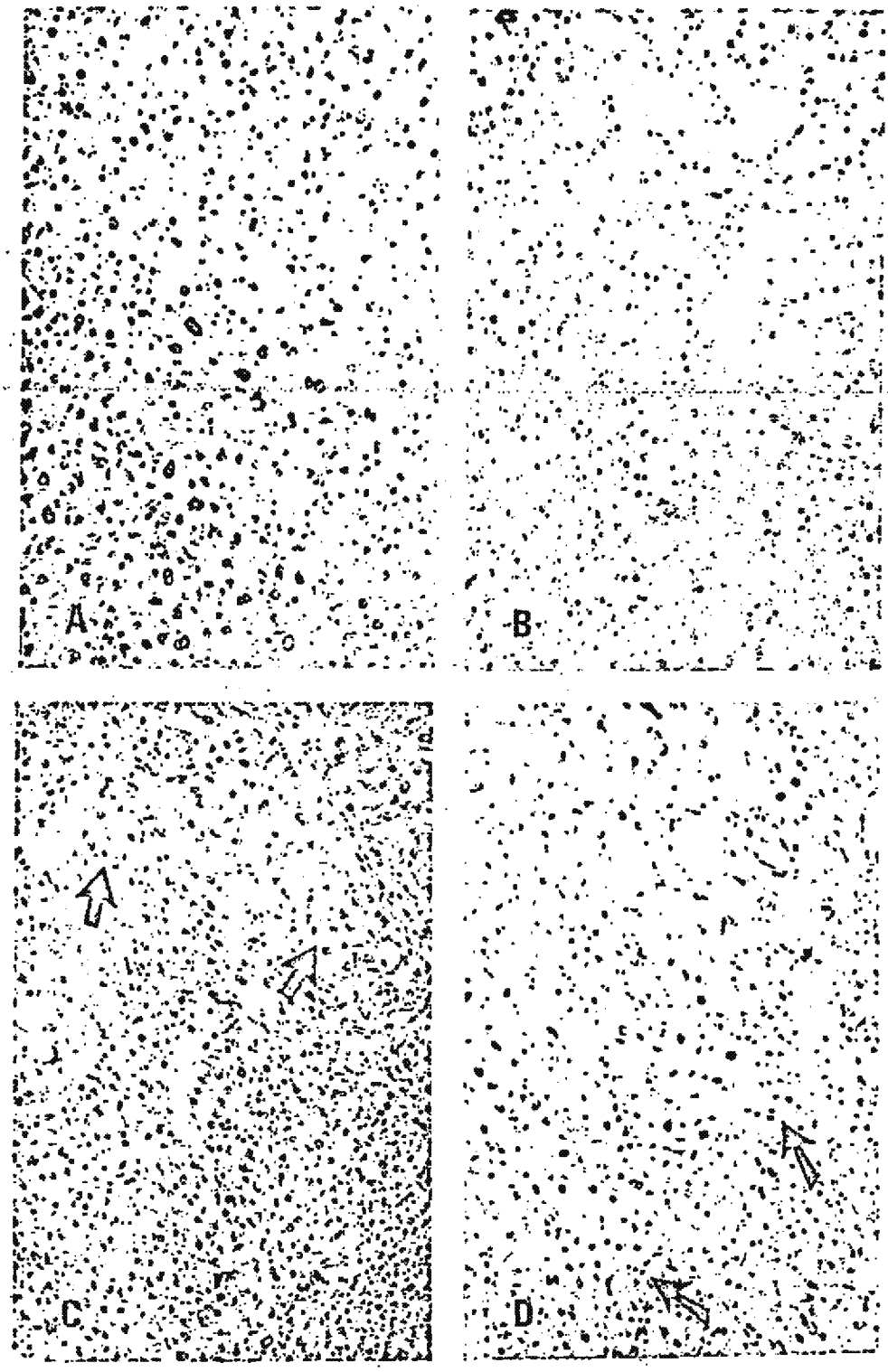
Various descriptions of the earlier lesions induced by vinyl chloride (16, 17) and of angiosarcoma (16, 18, 19, 20) in experimental animals have been published and the similarity of human and experimental lesions was referred to in a monograph (21). Therefore, the parallel sequences in the liver of man and of rodents (rats and mice) exposed to vinyl chloride were compared in order: (a) to review the pathologic features induced by vinyl chloride; (b) to document similarities in evolution from precursor lesions to malignant states produced by the same agent in man and experimental animals, rarely available with other agents, and thus to enforce the extrapolation from experimental hepatic tumors to man; (c) to compare the evolution with that caused by other carcinogenic agents, thus contributing to the study of carcinogenesis in general; (d) to provide histologic criteria for precursor changes which may also be important in the recognition of the effect of agents other than the ones mentioned above; such agents, particularly those of industrial nature, might be incriminated in the future; and (e) to point out features of general pathobiologic significance.

Material and methods

Available for study were 19 human cases of angiosarcoma and precursor lesions following exposure to vinyl chloride, previously reported (4), plus 5 additional angiosarcomas and 16 precursor lesions, the former being autopsy, the latter biopsy material. The rats and mice had been exposed to vinyl chloride in inhalation chambers at the Bologna Institute of Oncology, Italy. Details of the number of animals and duration of exposure have been given in several publications (2, 22, 23). Paraffin blocks from 11 rats and 4 mice were available for detailed study. This material was supplemented by the tissue of 46 mice exposed to 2500–6000 ppm vinyl chloride for 5 h a day, 5 days a week. The animals were sacrificed after 1–6 months of exposure (24).

All material, fixed in buffered formalin, was studied by routine light-microscopic tech-

Fig. 1. A. Worker exposed to vinyl chloride. Focal variations of hepatocytes in size of cytoplasm and in nuclear appearance. Some nuclei are large and polychromatic or show inclusions. H & E, 240×. *B.* Female rat exposed to 500 ppm vinyl chloride for 120 weeks. Nodule consisting of basophilic hepatocytes which are larger than in the surrounding parenchyma and are arranged in more than one-cell-thick plates; the surrounding parenchyma shows some sinusoidal dilatation, and the hepatocytic plates bordering on the nodules are slightly compressed. H & E, 100×. *C.* Female rat exposed since birth to 500 ppm vinyl chloride for 76 weeks. Trabecular hepatocellular carcinoma in upper aspect (arrows), revealing moderate degree of anaplasia. H & E, 100×. *D.* Vinyl chloride worker. Part of a nodular portion exhibiting hyperplasia of both hepatocytes and sinusoidal cells. The border towards the surrounding noncompressed parenchyma is indicated by arrows. H & E, 240×.



niques, including hematoxylin eosin stain, silver impregnation with reduced toning to provide a yellow color for hard, nonreticulin collagen, PAS reaction with or without preceding digestion by diastase, aniline blue connective-tissue stain, elastica stain with orcein, and iron reaction.

Results

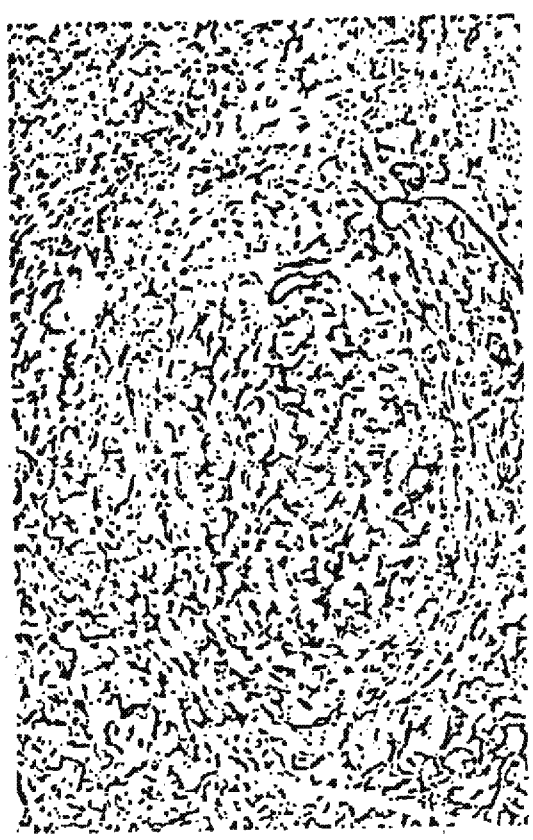
To demonstrate the similarity in evolution between human and animal lesions, the stages are discussed together and differences between man and rodents are pointed out.

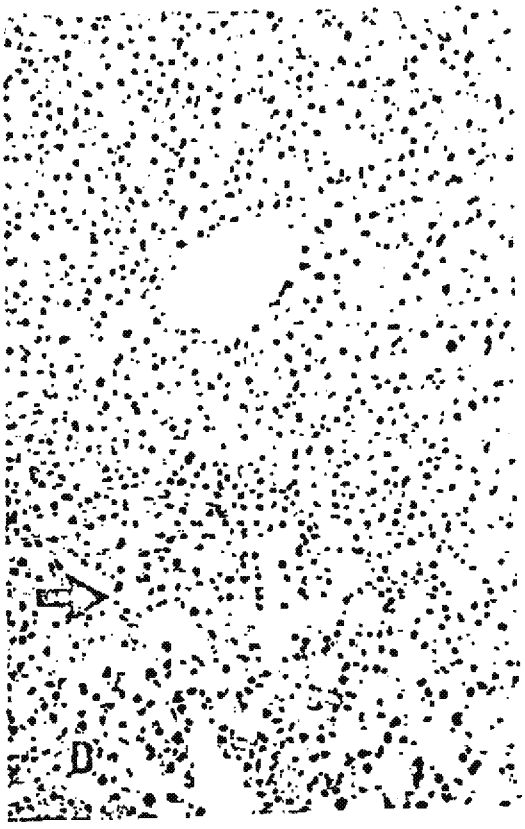
Precursor lesions

(a) *Focal hyperplasia and hypertrophy of hepatocytes.* In man, hepatocytes in barely demarcated areas exhibited conspicuous variations in size of cytoplasm and nuclei. Small cells contrasted to large cells and the staining qualities of the nuclei varied (Fig. 1A). In rodents, circumscribed nodular zones were noted in which the hepatocytes were larger and arranged in plates two or more cells thick. The staining of the cytoplasm was slightly darker but otherwise did not differ from the surrounding parenchyma, on which no pressure was exerted. Most nodules were not larger than a lobule or acinus and only slightly disturbed the parenchymal architecture (Fig. 1B). Nodules with basophilic cytoplasm larger than a lobule were also found and some were even grossly visible. In rats exposed at birth to inhalation of vinyl chloride, frank hepatocellular carcinoma developed (Fig. 1C). The hepatocytes were then arranged in three-(or more)-cell-thick plates and were anaplastic. The reticulin framework was not thickened but the sinusoids were conspicuously dilated in the tumor as well as in the surrounding parenchyma (see later).

(b) *Areas of combined hyperplasia and hypertrophy of hepatocytes and sinusoidal cells.* In man, often in the presence of angiosarcoma somewhere else in the liver, the enlarged hepatocytes were arranged in two-cell-thick plates and the sinusoidal cells were increased in number. They consisted of a variety of cells, some of them enlarged endothelial cells, others lymphocytes, and many were macrophages with PAS-positive diastase-resistant granules, while some appeared to be fat-storing cells (Fig. 1D). These mixed nodules were smaller than lobules, often garland-shaped, and frequently extended in bent fashion toward the portal tracts. Some involved contiguous portions of lobules. The surrounding parenchyma might show compression, increased lipofuscin and sometimes cholestasis. The reticulin framework in these nodular areas was distinctly increased (Fig. 2A). A noncharacteristic portal fibrosis was often present, as were nodular accumulations of subcapsular connective tissue in surgical and autopsy specimens. In rodents, conspicuous hypertrophy and hyperplasia of the hepatocytes were seen in nodules in which the sinusoidal lining cells were activated (Fig. 2B). Again, the reticulin framework appeared markedly in-

Fig. 2. A. Vinyl chloride worker. Increased reticulin framework in a nodular portion in center of field. Silver impregnation, 100×. *B.* Female mouse exposed to 6000 ppm for 41 weeks. The hepatocytes in the nodular portion in the upper aspect of the field are larger, more basophilic, arranged in two-cell-thick plates and are surrounded by increased sinusoidal cells which include hematopoietic cells (arrow). Pressure is not exerted on the surrounding parenchyma, and transition from normal to hyperplastic cells is noted in some hepatocellular plates. H & E, 100×. *C.* Female rat exposed to 500 ppm vinyl chloride for 76 weeks. Increased reticulin framework in a nodule in center of field, which contrasts with the sparse reticulin in the surrounding parenchyma. Silver impregnation, 100×. *D.* Vinyl chloride worker. Circumscribed dilatation of sinusoids not related to the hepatic vein tributaries; the hepatocytes in the area of dilatation appear hyperplastic (arrow), sometimes in two-cell-thick plates; note slight activation of sinusoidal cells. H & E, 100×.





creased and permitted demarcation from the surrounding parenchyma (Fig. 2C).

(c) *Sinusoidal dilatation.* As a rule, in the presence of the lesions described in (a) and (b), but not necessarily in the same area, sinusoids were conspicuously dilated in both man and rodents. This was noted in ill-defined portions of the hepatic lobules. In contrast to passive congestion, the hepatic plates in such areas were not narrowed and were sometimes more than one cell thick (Fig. 2D). The sinusoidal lining cells appeared increased and enlarged and the reticulin framework was not rarified but was often increased. Groups of hematopoietic cells (nucleated erythrocytes and occasional megakaryocytes) were often found.

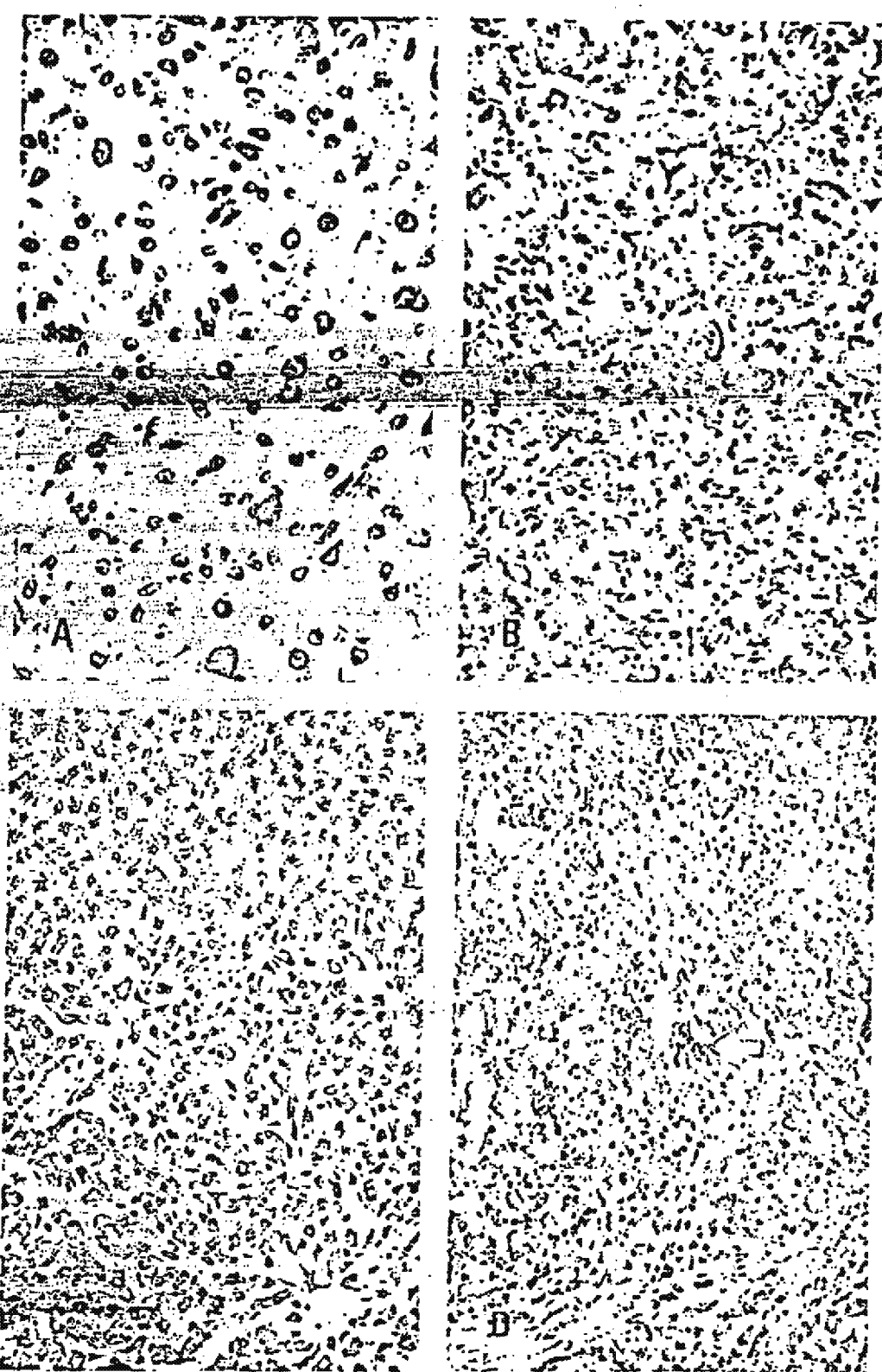
Transformation to angiosarcoma

The transition to angiosarcoma in all species may be associated with sinusoidal dilatation; in its absence, intralobular angiosarcoma formed, while in its presence the more frequent trabecular form developed.

(1) *Transition to intralobular angiosarcoma.* In man, the number of sinusoidal cells conspicuously increased and they almost filled the spaces between the enlarged hepatocytes. They were the various cells described above, but now also included segmented leukocytes (Fig. 3A). Among them were lining cells with large polychromatic nuclei, which predominated in, apparently, later stages and eventually were the majority of lining cells which were often arranged in multiple layers (Fig. 3B). The reticulin framework was conspicuously increased to form a thick, contiguous membrane which also contained bundles of hard, non-reticulin collagen. In places the fibroplasia was excessive; the squeezed hepatocytes appeared atrophic and eventually disappeared, so that broad areas of fibrous tissue with hard-collagen bundles and elastic fibers contained few hepatocytes in pseudoductular arrangement and some angiosarcoma cells which might line vascular spaces. Moreover, foci of ischemic necrosis were seen, seemingly the result of the disturbance of microcirculation. In rodents, the variety of sinusoidal cells was less conspicuous and fewer macrophages were found, but lining cells with large polychromatic nuclei similarly predominated (Fig. 3C). However, the reticulin framework was only slightly increased, hard-collagen bundles were rare, and elastic fibers were absent. In both man and rodents, the intralobular angiosarcoma involved grossly visible portions of the liver.

(2) *Transition to trabecular angiosarcoma.* In man and rodents, progression of the sinusoidal dilatation associated with proliferation of the hepatocytes and sinusoidal cells loosened the parenchymal architecture (Fig. 3D). Subsequently, hepatocytes in mostly two-cell-thick plates formed cords (Fig. 4A) which were surrounded by a thick coat of reticulin and were lined by layers of sarcoma cells with varying degrees of anaplasia (12). These cords were invaded by angiosarcoma cells. The fibroplasia seemed to increase, so that the hepatocytes were eventually replaced by fibrous tissue, and

Fig. 3. A. Vinyl chloride worker. Conspicuous increase of sinusoidal and perisinusoidal cells greatly filling the sinusoids; these cells vary in character and a few are anaplastic. H & E, 240×. *B.* Vinyl chloride worker. Intralobular angiosarcoma in that tumor cells fill the sinusoids and are often arranged in several layers. H & E, 100×. *C.* Female rat exposed to 30,000 ppm vinyl chloride for 51 weeks. Intralobular angiosarcoma in lower field. Note anaplastic cells, partly filling the sinusoids. H & E, 100×. *D.* Vinyl chloride worker. Dilatation of sinusoids loosens the parenchymal architecture. The hepatocytes are hyperplastic; in and around sinusoids many mesenchymal cells are noted, including hematopoietic cells (arrow). H & E, 100×.



trabecles formed consisting of mainly hard collagen and elastic fibers (Fig. 4B). As the blood spaces expanded further, they were traversed by beams consisting of a few hepatocellular cords, persisting portal tracts and central canals, all surrounded by dense collagenous tissue and infiltrated by angiosarcoma cells (Fig. 4C). In earlier stages in man, the hepatocytic plates frequently contained bile plugs in dilated bile canaliculi and were surrounded by widened perisinusoidal (Disse) spaces full of various cells and increased reticulin (Fig. 5A). In rodents, the lining angiosarcoma cells were frequently a homogeneous stratum of polyhedral cells without inflammatory cells (Fig. 4D).

Features of fully developed angiosarcoma

Nodules consisting of angiosarcoma cells were noted in man and rodents. Most consisted of spindle-shaped cells, in part in vascular spaces which, however, in part were also lined by nontumorous endothelial cells. *In addition, in man,* nodules consisted of large polyhedral sarcoma cells with abundant eosinophilic cytoplasm. The center of these nodules usually exhibited hemorrhagic necrosis and these cells then lined bloody cysts (Fig. 5B). However, similar cysts were more frequently the result of sinusoidal dilatation or disturbance of circulation in man and rodents. In all species, venous invasion was common. Another feature, so far observed only in man, was primary angiosarcomatous growth in portal tracts, although secondary invasion of portal tracts was frequent in man and rodents. Abnormal bile duct epithelium was seen only in man.

At autopsy, angiosarcoma with extensive fibrosis, necrosis and hemorrhage was always multicentric in man and rodents, although it was sometimes restricted to one lobe. Cirrhosis was never encountered.

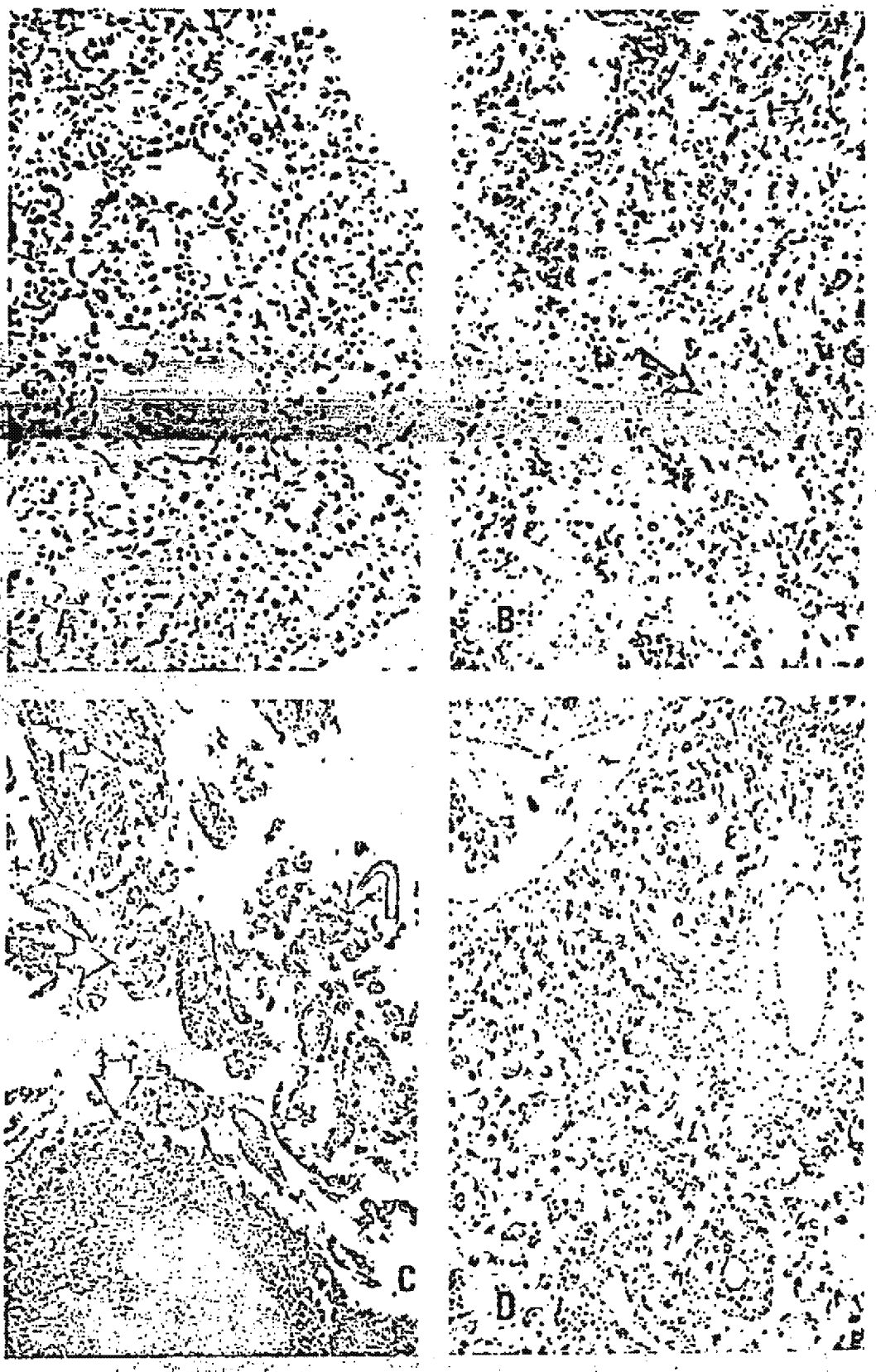
Discussion

The hepatic lesions associated with vinyl chloride in man and rodents exhibit an identical sequence from proliferation of hepatocytes and of sinusoidal cells, frequently associated with sinusoidal dilatation, to angiosarcoma as well as, at least in rodents, to hepatocellular carcinoma. Obstruction of sinusoidal spaces in the intralobular angiosarcoma leads to ischemic necrosis, and a characteristic loosening of the parenchymal architecture in the more frequent trabecular form causes bloody cysts or peliosis.

The demonstrated similarity of the evolution in man and rodents strongly supports the extrapolation of observations from experimental animals to man. Only a few morphologic differences are apparent. They include:

(1) The lesser degree of inflammatory reaction and of fibrosis in rodents, in which excess elastic fibers do not form and hyalinization of connective tissue is less conspicuous.

Fig. 4. A. Vinyl chloride worker. Trabecular angiosarcoma. Note cords of hepatocytes, usually in more than one-cell-thick plates, lined by angiosarcoma cells and surrounded by conspicuously dilated vascular spaces. H & E, 100×. *B.* Vinyl chloride worker. Trabecular angiosarcoma; extensive fibroplasia replaces hepatocytes (arrow), which are in part hyperplastic, in part atrophic; angiosarcoma cells are also in trabecles. H & E, 100×. *C.* Vinyl chloride worker. Far advanced trabecular angiosarcoma with excessively dilated blood spaces. Except for clots (thick arrow), the blood has fallen out during processing; the spaces are traversed by hepatocytic cords (curved arrow) and by remnants of portal and central canals (slender arrows); all are lined and infiltrated by angiosarcoma cells. H & E, 40×. *D. Female mouse exposed to 10,000 ppm* vinyl chloride for 62 weeks. Trabecular angiosarcoma; polyhedral sarcoma cells line and invade remnants of portal and central canals which traverse blood spaces. H & E, 100×.



(2) Initiation of the angiosarcomatous process in portal tracts has not been observed in rodents.
(3) Nodules of polyhedral sarcoma cells were only observed in man.
(4) While in rodents hepatocellular carcinoma is frequent after exposure in the neonatal period (23), and was also observed after exposure in maturity, although rarely (16, 19, 23), very few cases of hepatocellular carcinoma were observed in man (25). In one patient, without established etiology, both hepatocellular carcinoma and angiosarcoma were noted (4).
(5) Minor light-microscopic changes following exposure to vinyl chloride were observed in animals many years ago (26, 27, 28) and more recently also in man (29), although not by us. They have been confirmed by electron microscopy in animals (16, 20, 30) as well as in man (29, 31). However, extensive acute hepatocellular necrosis has only been demonstrated in rats when large doses of vinyl chloride were administered in combination with phenobarbital and methylcholanthrene, which increase the activity of microsomal enzymes (32, 33). The lesion is explained by an increased formation of epoxide not sufficiently inactivated by epoxide hydrase or by binding to glutathione (34).
(6) In rodents, tumors in other organs have been observed, particularly, alveolar adenoma of the lung (2, 18, 22, 35) in high incidence.

The similarity of the basic sequences between rodents and man has several implications:
(a) Dose dependency of both precursor lesions and particularly of angiosarcoma can be clearly demonstrated in experimental animals (18, 20, 23, 36). Indeed, the production of angiosarcomas by relatively small doses of vinyl chloride in rodents has served the regulatory agencies in establishing permissible concentrations of vinyl chloride in the ambient air of factories. This reduces the chance of additional initiation of angiosarcomas in exposed workers. Moreover, the risk of angiosarcomas in workers exposed to vinyl chloride has been calculated from studies in rats (37, 38).
(b) The increased sensitivity of young animals (20, 23) may apply to man.
(c) Electron-microscopic studies to identify the nature of the tumor cells have been carried out primarily in rodents (24) and suggest an endothelial origin of the tumor cells. This is so far best confirmed by the use of factor VIII as a marker for endothelial cells (39) in vinyl chloride-induced angiosarcoma (40).
(d) Increased incidence of tumors in animals exposed to both ethanol and vinyl chloride (19) suggests a higher risk to workers who are alcoholics.
(e) The extensive investigations as to the metabolism of vinyl chloride in animals (34) [including monkeys (41)] may be applicable to man, as are the observations of potential mutagenicity (42).
(f) The fact that cirrhosis has not been observed in the examined human and animal material, although it may be associated with hepatic angiosarcoma of unknown etiology (13), speaks against vinyl chloride and related substances being an etiologic factor in human cryptogenic cirrhosis.
(g) Most important is the recognition of focal hepatocelluiar and mixed sinusoidal cell hyperplasia associated with increased reticulin as a precursor lesion. It thus served in the screening of workers (43). It might have practical significance since the common hepatic tests are not reliable, and either laparoscopy (5) or angiography is required. These precursor lesions appear to be characteristic of a group of environmental agents, including those produced by inorganic arsenicals, thorotrast, and anabolic/androgenic steroids (10). The lesion may become important in detecting lesions induced by industrial agents related to vinyl chloride, such as vinyl bromide (17) and vinylidine chloride (44). Angiosarcomas in experimental animals have been produced by a variety of other agents, which include dimethyl-

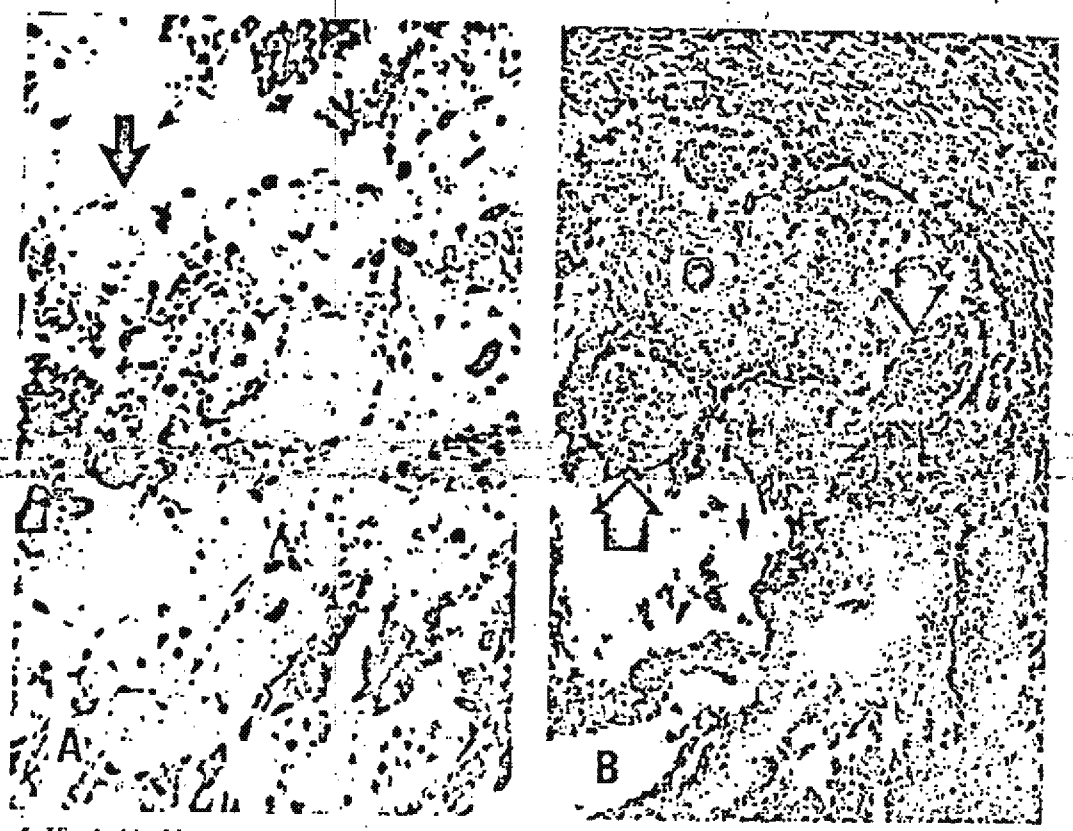


Fig. 5. Vinyl chloride workers. *A*. Hepatocytes in acinar arrangement often surround dilated bile canaliculi containing bile plugs; the tissue (Disse) spaces are widened and contain various perisinusoidal cells; the cords are lined by angiosarcoma cells. H & E, 240×. *B*. Trabecular angiosarcoma. Note bloody cysts lined by polyhedral sarcoma cells which also extend as homogeneous solid trabecles appearing as nodules on the cross-section (arrows) into the blood spaces. H & E, 40×.

nitrosamine, diethylnitrosamine, azoxymethanol (45), and N-nitroso-2,6-dimethylmorpholine (46).

The presented observations illuminate general pathobiological problems, namely, hepatic nodules in carcinogenesis, interaction of hepatocytes with sinusoidal cells and fibroplasia. Vinyl chloride produces in rodents hyperplastic or neoplastic hepatocellular nodules (16, 17) similar to those typically seen after administration of many agents, most of which are suspected of being carcinogenic. They also exhibit the characteristic deficiency in such enzymes as glucose-6-phosphatase and ATPase (47). They further show the characteristic variations in cell populations (48), or "nodules in nodules" (49), and may proceed to hepatocellular carcinoma. In contrast to the commonly seen hepatocytic nodules, the sinusoidal cells also proliferate following vinyl chloride exposure. In man, the increase in sinusoidal cells and the fibroplasia develop rapidly. In animals also, the parenchymal hyperplasia is followed by sinusoidal cell hyperactivity, reflected in increased alkaline phosphatase activity (16, 17), suggesting an action of the bioactive metabolite also on the sinusoidal cell, perhaps preferentially.

Whatever the metabolic basis for the specific reaction of each cell type (50), possibly depending on local variation in formation and degradation of metabolites, four types of com-

petition are suggested by the morphologic appearance. One is the proliferation of hepatocytes versus that of sinusoidal cells; thus, in some instances, angiosarcoma and, in others (mainly young animals), hepatocellular carcinoma develops. The second entails competition between proliferating hepatocytes and fibroplasia, which leads to the disappearance of the hepatocytes. The third is the simultaneous perisinusoidal fibrosis and sinusoidal dilatation resulting in a loosening of the architecture, progressing to peliosis; a sinusoidicidal effect has been assumed, at least for the peliosis following the administration of [illegible]. Finally, a diffuse initial proliferation of sinusoidal cells of various types, even including hematopoietic cells, is eventually replaced by predominant sarcoma cells. The role of this initial inflammatory reaction and its disappearance requires elucidation. An immunologic reaction has been assumed in vinyl chloride disease (52), and, interestingly, while antilymphocytic serum does not influence development of hepatocellular carcinoma in animals treated with azoxymethane, angiosarcoma formation is favored (45). The polyhedral sarcoma cells resemble the recently described histiocytoid tumor cells (53), which so far have not been described in the liver and apparently have a lower malignant potential.

The abundance of fat-storing, or Ito, cells in the precursor lesion (31, 54, 55) may support their fibroblastic potential (56). The fibrosis is far more conspicuous in man than in rodents. This holds true not only for reticulin fibers but even more so for the formation of hard collagen, presumably type I, and for the fibers giving elastica reaction. Interestingly, the normal rat liver contains one-third as much collagen as the human liver, and fully developed rat cirrhosis has a collagen content by unit weight which is hardly higher than the normal human liver (57).

As stated some years ago (58), human environmental pathology supplemented by animal studies has great heuristic value by providing, from relatively few instances, lessons to pathobiology.

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